

AMENDMENTS TO THE SPECIFICATION:

Please replace the paragraph beginning "Using the Escherichia coli" at page 17, line 15, with the following amended paragraph:

Using the Escherichia coli expression system described by Shen, et al. (1993); U.S. Patent No. 5,753,465; and Kim, et al. (1995); U.S. Patent No. 5,843,888, new non-naturally occurring artificial recombinant hemoglobins ("rHbs") have been constructed, having low oxygen affinity while maintaining high cooperativity in oxygen binding. One of the rHbs, rHb ( $\beta$ N108Q) also exhibits increased resistance to autoxidation as compared to certain other known low oxygen affinity mutants. More particularly, the present invention is directed to: a recombinantly produced mutant of Hb A, denoted herein as rHb ( $\beta$ N108Q), in which the asparagine residues at position 108 of each of the  $\beta$ -chains (SEQ ID NO: 8), located in the  $\alpha_1\beta_1$  subunit interface and in the central cavity of the Hb molecule, have been replaced by glutamine residue; and a recombinantly produced mutant of Hb A, denoted herein as rHb ( $\beta$ L105W) in which the leucine residues at position 105 of each of the  $\beta$  chains (SEQ ID NO: 8) have been replaced by tryptophan and in this molecule a

by stabilizing its deoxy quaternary structure.

Please replace the paragraph beginning "The construction of plasmid pHE7009" at page 25, line 5, with the following amended paragraph:

The construction of plasmid pHE7009 for expression of mutant rHb ( $\beta$ N108Q) using the human globin cDNAs was carried out as follows. The coding sequences of human  $\alpha$ - and  $\beta$ -globin cDNAs in plasmid pHE7 were inserted into pTZ18U (Bio-Rad Laboratories, Hercules, CA) by methods well known in the art. Site-directed mutagenesis was performed as described by Kunkel, T.M. et al., Proc. Natl. Acad. Sci. USA 82:488 (1985) the disclosures of which are incorporated herein by reference, and Shen, et al. (1993). An oligonucleotide of sequence 5'-ACAGACCAGTACTTGTCCCAGGAGCCT-3' (SEQ ID NO: 4) (mutated codon Asn $\rightarrow$ Gln is underlined) was purchased from DNA International, Inc. (Lake Oswego, Oregon), and used as the mutation primer. The human normal  $\beta$ -globin cDNA in plasmid pHE7 was then replaced with the mutated cDNA to produce plasmid pHE7009. The DNA sequences for the  $\alpha$ - and  $\beta$ -globin cDNAs in pHE7009 are shown in Figure 1A (SEQ ID NO: 5). The amino acid sequence for the human beta chains of hemoglobin is shown in SEQ ID NO: 8. Plasmid pHE7009 in host cell E. coli JM109 and designated pHE7009/JM109 was deposited with the American Type

Please replace the paragraph beginning "The construction of plasmid pHE7004" at page 25, line 20 with the following amended paragraph:

The construction of plasmid pHE7004 for expression of mutant rHb ( $\beta$ L105W) using the human globin cDNAs was carried out in the similar way as that of plasmid pHE7009, except an oligonucleotide of sequence 5'-CCTGAGAACTTCAGGTGGCTAGGCAACG TGCTGGTC-3' ((SEQ ID NO: 6), mutated codon Leu→Trp is underlined) was purchased from DNA International, Inc. (Lake Oswego, Oregon) and used as the mutation primer. The DNA sequences of the  $\alpha$ - and  $\beta$ -globin cDNAs in pHE7004 are shown in Figure 1B (SEQ ID NO: 7). The amino acid sequence for the human beta chains of hemoglobin is shown in SEQ ID NO:8. Plasmid pHE7004 in host cell E. coli JM109 and designated pHE7004/JM109 was deposited with the American Type Culture Collection of Manassas, VA on April 27, 2000 under number PTA-1769.

Please replace the previously filed Sequence Listing with the supplemental Sequence Listing filed herewith.